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MACROPHAGE-DERIVED CYTOKINE AND NUCLEAR FACTOR κB p65 EXPRESSION IN SYNOVIAL MEMBRANE AND SKIN OF PATIENTS WITH PSORIATIC ARTHRITIS

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Objective. **Monocyte-derived cytokines are important mediators in synovitis and represent novel therapeutic targets. This study was undertaken to analyze their expression in synovial membrane (SM) of patients with psoriatic arthritis (PsA) compared with that in skin of patients with PsA and SM of patients with rheumatoid arthritis (RA).**

Methods. **Multiple synovial biopsy samples (24 from patients with PsA, 20 from patients with RA, 5 from patients with osteoarthritis [OA]) and skin biopsy samples (lesional and perilesional skin from 25 PsA patients) were obtained. Standard leukocyte antigens, cytokines (tumor necrosis factor α [TNFα], interleukin-1α [IL-1α], IL-1β, IL-15, and IL-10) and the transcription factor nuclear factor κB (NF-κB; active p65 subunit) were localized and quantified immunohistochemically by light microscopy and digital image analysis.**

Results. **Sublining cellular infiltration, lymphoid aggregation, and vascularity were similar in PsA and RA SM. Lining layer thickness was greater in RA SM, associated with more CD68+ macrophages. In PsA SM, TNFα, IL-1α, IL-1β, IL-15, and IL-10 were primarily localized to lining layer and perivascular macrophages, as were cells expressing the active subunit of NF-κB (p65). TNFα, IL-1β, and IL-15 expression in PsA lining layer was less than that in RA lining layer, likely reflecting lower macrophage numbers. In sublining areas, levels of TNFα and IL-15 were lower in PsA patients than in RA patients, whereas IL-1α and IL-1β expression was equivalent. IL-10 was identified at similar levels in RA and PsA SM lining layer and sublining. Expression of NF-κB (p65) was equal in lining layer from both patient groups, but lower in PsA than RA sublining. Histologic findings did not correlate with clinical parameters of disease. Cytokine expression in skin did not correlate directly with that in SM. Cytokine expression was greater in PsA and RA SM than in OA SM.**

Conclusion. **This study shows, for the first time, that monocyte-derived cytokines are found in PsA SM and demonstrates the relative paucity of the antiinflammatory cytokine IL-10 in PsA skin and SM. Significant divergence from RA SM expression was observed, despite similar clinical and demographic features in the 2 patient groups.**

The pathogenic role of macrophage-derived cytokines has been established in a variety of chronic inflammatory diseases. Moreover, their clinical utility as therapeutic targets has been demonstrated through successful targeting of tumor necrosis factor α (TNFα) in rheumatoid arthritis (RA) and inflammatory bowel disease (1–3). Whether such observations can be extended to other rheumatic diseases is unproven. A prerequisite will be the characterization of cytokine expression in involved tissues.

With a prevalence of ~2% in the North American population, psoriasis is a common chronic inflammatory skin disease. Psoriatic arthritis (PsA), an inflammatory arthropathy of varied phenotype which develops in 5–7% of patients with psoriasis, may be associated with significant articular destruction and subsequent morbidity (4). Simultaneous involvement of skin and joints provides a unique opportunity to study the inflammatory response in 2 distinct tissues. Common histologic

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features at the 2 sites have been described, including activation and expansion of tissue-specific cell subsets (i.e., keratinocytes and synoviocytes), infiltration by mononuclear cells, and angiogenesis (4–7). Moreover, the results of several studies indicate a pathologic role for T cells (suggested to initiate the inflammatory response) and involvement of macrophages and synoviocytes/keratinocytes in amplifying and perpetuating the effector phase at both sites (6–11). Whether cytokine expression or its functional significance in the 2 tissues is similar, however, is currently unclear, and this has major implications for the prediction of the likely relative efficacy of biologic agents.

Comparative studies between PsA synovial membrane (SM) and RA SM suggest the presence of altered vascularity and adhesion molecule expression and reduced lining layer expansion in PsA (12). Although TNFα and interleukin-1 (IL-1) have been detected by enzyme-linked immunosorbent assay in PsA synovial fluid (13), relatively little is known about the extent, distribution, and correlation of their expression in PsA skin and synovial tissue, or about how such expression compares with that in RA synovium.

In the present study, we systematically characterized the histopathologic appearance and expression of key pro- and antiinflammatory cytokines in SM biopsy specimens from PsA patients and compared the results with the findings in SM obtained from a cohort of RA patients with equivalent demographic and disease activity characteristics. In addition, we performed comparisons between matched samples of involved PsA skin versus PsA SM, and osteoarthritis (OA) SM versus PsA SM. Our data suggest that TNFα, IL-1α, IL-1β, IL-15, and IL-10 are indeed expressed both in skin and SM in PsA, at higher levels than in a noninflammatory arthropathy such as OA. The distribution of monokine expression appeared similar in RA and PsA SM, although quantitative differences were evident. Differences were also noted in the relative expression of an active subunit of the transcription factor nuclear factor κB (NF-κB), which is activated by proinflammatory cytokines such as TNFα. These observations suggest that, while immunopathogenic responses in PsA and RA SM may be broadly similar, there are subtle but potentially functionally important differences.

PATIENTS AND METHODS

Patients. Patients were seen in the Rheumatology Day Hospital at the National Institutes of Health. Each of the PsA patients had a history of inflammatory peripheral arthritis (with or without spinal involvement) and characteristic psoriatic skin disease of at least 6 months' duration. All PsA patients were rheumatoid factor (RF) negative and had received no standard disease-modifying agents, other than low-dose prednisone (≤10 mg daily), within 4 weeks of evaluation and biopsy. Prednisone was withheld for 24 hours prior to biopsy. Patients had active disease, defined as at least 3 swollen and tender peripheral joints and morning stiffness for >45 minutes, with or without an elevated erythrocyte sedimentation rate (>28 mm/hour) or C-reactive protein level (≥0.8 mg/dl). The RA patients included in the study met the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 criteria for RA (14), were RF positive, and likewise had active disease as defined above. Five of the RA patients were taking either methotrexate or sulfasalazine.

Fifty-four patients (34 PsA, 20 RA) were recruited and underwent an assessment that included clinical history and examination, radiologic evaluation, laboratory studies, and HLA typing. After informed consent was obtained, the most actively inflamed and accessible joint was biopsied using a blinded closed-needle technique. Multiple synovial fragments were obtained and mounted in OCT freezing medium (3 fragments/block; subsequent analysis revealed evaluable samples available as follows: single biopsy 24%, 2 biopsies 40%, 3 biopsies 36%).

Synovial tissue samples were obtained at the time of surgical synovectomy in 13 of the RA patients and by needle biopsy in the other 7. In 25 PsA patients with accessible involved skin, 3-mm punch biopsies of psoriatic plaques and perilesional, uninvolved skin were obtained as processed as above. Synovial tissue samples were also obtained at arthroplasty from 5 patients with OA diagnosed based on clinical and radiologic findings, RF negativity, and absence of acute-phase response.

Of the 54 PsA and RA patients evaluated, SM samples from 10 (all with PsA) were not evaluable. Therefore, histologic analysis was performed on the remaining 44 samples (24 PsA and 20 RA).

Immunohistochemistry studies. Eight-micrometer sections were fixed on gelatin-coated slides in chilled acetone, then air dried. The following monoclonal antibodies (mAb) were used: CD3, CD8, CD20, CD68, von Willebrand factor (vWF) (all from Dako, Carpinteria, CA), CD14, TNFα (both from Becton Dickinson, San Jose, CA), IL-1α (Calbiochem, La Jolla, CA), IL-1β (Genzyme, Cambridge, MA), IL-15 and IL-10 (R&D Systems, Minneapolis, MN), and NF-κB p65 subunit (Santa Cruz Biotechnology, Santa Cruz, CA). Tissue sections were rehydrated in Tris buffered saline and then pretreated with 0.03% hydrogen peroxidase blocking solution. Slides were blocked in 25% normal goat serum/25% pooled human serum, after which 100 μl of diluted primary mAb was applied overnight at 4°C in a humidified slide chamber. Appropriate secondary biotinylated immunoglobulin, streptavidin–horseradish peroxidase, and diaminobenzidine substrate were utilized to detect binding. Control procedures included use of isotype-matched murine mAb of irrelevant specificity and/or absence of primary antibody. To confirm specificity of cytokine staining, primary antibodies were preincubated with recombinant cytokine (R&D Systems) for 60–120 minutes at 37°C prior to submission to the above protocol.

Table 1. Patient clinical characteristics at study enrollment*

Variable	PsA patients (n = 24)	RA patients (n = 20)
No. male/no. female	14/10	2/18
Age, years	40.7 ± 11.3	50.1 ± 10.8†
Disease duration, years	12.2 ± 11.0	14.3 ± 9.1
Tender joint count	22.3 ± 11.2	25.1 ± 8.6
Tender joint score	37.5 ± 21.0	39.6 ± 17.0
Swollen joint count	17.0 ± 9.2	22.8 ± 5.3
Swollen joint score	24.0 ± 16.0	29.7 ± 6.6
% with erosions	80	100
% with new bone formation	44	NA
% with enthesitis/dactylitis	56	NA
% with spinal involvement	44	NA
PASI score	6.5 ± 6.4	NA
HAQ score	0.7 ± 0.4	1.4 ± 0.5†
ESR, mm/hour	38.5 ± 24.7	63.5 ± 23.7‡
CRP, mg/dl	1.2 ± 1.1	4.9 ± 3.3‡
% taking corticosteroids (<10 mg prednisone/day)	21	68

* Except where otherwise indicated, values are the mean ± SD. PsA = psoriatic arthritis; RA = rheumatoid arthritis; NA = not applicable; PASI = Psoriasis Area and Severity Index (45); HAQ = Health Assessment Questionnaire (46); ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.
† $P < 0.04$ versus PsA group.
‡ $P \leq 0.001$ versus PsA group.

Quantification. Quantification was performed by light microscopy on an Olympus BX60 optic system. SM staining was assessed in lining layer and sublining areas separately, as was epidermal and dermal staining in skin. Cytokine expression was low in aggregate areas and proved impossible to reliably quantify because high cell density precluded assignment of cytokine staining to individual nuclei. A mean of 14 (range 12–20) high-power fields (hpf) at 400× magnification were analyzed independently for each sample by 2 observers, who were blinded as to the patient's demographic characteristics and diagnosis. Cytokine-positive cells were reported as a percentage of total nuclei per hpf. Skin epidermal areas were calculated in square microns by computer image analysis using Scion (Gaithersburg, MD) imaging software. Significant interobserver discrepancy occurred in <10% of samples and was addressed by reevaluation and achievement of consensus.

Statistical analysis. Group medians or means ± 1 SD (of the mean) are reported. Data were analyzed using Student's *t*-test or, when variables demonstrated a nonparametric distribution, Mann-Whitney U test. Correlations were tested using linear regression. Because this was a hypothesis-generating, descriptive study and the target cytokines were specified a priori, *P* values were not adjusted for multiple comparisons. *P* values less than 0.05 were considered significant.

RESULTS

Clinical data. To facilitate interpretation of the histologic findings, it was important to properly characterize the clinical cohorts. Patient characteristics are shown in Table 1. The PsA group was composed mainly of patients with polyarthritis (23 of 24 patients; the remaining patient had oligoarthritis) with large and small joint involvement. The mean ± SD duration of articular involvement in the PsA group was 12.2 ± 11.0 years, which was comparable with that in the RA group (14.3 ± 9.1 years). Similarly, disease activity was equivalent in the 2 groups, with no significant differences in tender and swollen joint scores. Eighty percent of the PsA patients had erosive disease seen on plain radiographs (4 had arthritis mutilans), and 44% had evidence of new bone formation. A subset of PsA patients (56%) had enthesitis (tenderness/swelling at the Achilles tendon insertion) and/or dactylitis of 1 or more digits. The PsA group included more males, and fewer PsA patients were taking low-dose prednisone (21%, versus 68% of the RA patients).

Histologic findings. Morphologic analysis of PsA SM demonstrated mild lining layer hyperplasia (median 2.1 cells thick, SD 0.5, range 1–5 cells) and diffuse sublining mononuclear cell infiltration (median cell count 77.8 total nuclei/hpf, SD 19.4) (Table 2). Twelve of 24 PsA patients and 10 of 20 RA patients had at least 1 dense cellular aggregate in the SM containing macrophages, T cells (CD3+), and B cells (CD20+). Macrophages, detected using both CD14 and CD68 antibodies, were most prevalent around blood vessels and in the lining layer/sublining regions. PsA SM had fewer macrophages in the lining layer (median ± SD 52.4 ± 17.9% CD68+ cells/hpf) than did RA SM (82.1 ± 14.2%; $P = 0.006$). In the sublining, CD68+ and CD14+ macrophages were similar in number (34.5 ±11.8% CD14+ cells/hpf in PsA versus 42.7 ± 14.6% in RA; $P > 0.05$). As expected, vWF staining revealed extensive vascularization of PsA SM (median 6.9 ± 2.6 blood vessels/hpf). This was not, however, significantly different from that observed in RA SM (6.1 ± 1.9).

Median cellularity and vascularity scores were comparable between PsA SM and matched involved skin, obtained whenever accessible lesions were present. Lesional skin samples demonstrated greater epidermal thickening, dermal inflammatory cell infiltration, and vascularity than perilesional skin (Table 2). Though normal in external appearance, perilesional skin was found to have dermal macrophage numbers that were similar to those in involved skin, suggesting that increased cellularity may reflect increased T cell infiltration within lesions.

TNFα expression. We next sought to determine cytokine expression within PsA and RA synovia and to

Table 2. Tissue morphology and cell phenotype in PsA versus RA SM and in PsA lesional versus perilesional skin*

	PsA SM	RA SM	PsA lesional skin	PsA perilesional skin
Synovial membrane				
Lining layer thickness†	2.1 ± 0.5	3.2 ± 1.1‡		
Cellularity count§	77.8 ± 19.4	90.8 ± 25.9		
Vascularity score¶	6.9 ± 2.6	6.1 ± 1.9		
CD3 count, sublining#	11.3 ± 14.2	19.7 ± 8.6‡		
CD14 count#				
Lining layer	36.4 ± 13.4	44.1 ± 20.0		
Sublining	34.5 ± 11.8	42.7 ± 14.6		
CD68 count				
Lining layer#	52.4 ± 17.9	82.1 ± 14.2‡		
Sublining**	3.0 ± 0.6	3.2 ± 0.6		
PsA skin				
Epidermal thickness††			6,700 ± 1,916	2,785 ± 1,068‡‡
Cellularity score§			70.3 ± 17.7	42.8 ± 6.3‡‡
Vascularity score¶			5.6 ± 1.5	4.3 ± 1.2‡‡
CD3 count, dermis#			54.4 ± 17.3	19.9 ± 7.9‡‡
CD14 count			ND	ND
CD68 count, dermis**			3.3 ± 0.2	3.2 ± 0.8

* PsA = psoriatic arthritis; RA = rheumatoid arthritis; SM = synovial membrane; ND = not determined.
† Median ± SD number of cells thick.
‡ $P \leq 0.01$ versus PsA SM.
§ Median ± SD total nuclei per high-power field (hpf).
¶ Median ± SD number of von Willebrand factor–positive blood vessels per hpf.
# Median ± SD % positive cells per hpf.
** Median ± SD score on a 0–4 scale: 1 = ≤10% cells positive, 2 = 11–25% positive, 3 = 26–50% positive, 4 = >50% positive.
†† Median ± SD epidermal thickness (μ^2) per hpf.
‡‡ $P \leq 0.001$ versus lesional skin.

compare this with expression in matched skin biopsy specimens. TNFα was prominently expressed in the lining layer of PsA SM, with a median ± SD of 7.1 ± 12.5% positive cells/hpf. Expression of TNFα by sublining inflammatory cells was also detected (4.2 ± 3.3% positive cells/hpf), particularly around blood vessels and including endothelial cells (Figure 1a). The pattern of cytokine staining in lining layer and perivascular cells was similar to that seen with CD68 staining, suggesting that TNFα expression was located primarily in macrophages. Levels of TNFα expressed in PsA SM did not correlate significantly with disease duration, disease activity parameters, or presence of erosions. Of note, fewer TNFα-positive cells were found both in the lining layer and sublining of PsA SM when compared with RA SM (RA lining layer 19.8 ± 12.0% positive cells/hpf [$P = 0.03$]; RA sublining 6.5 ± 9.7% [$P < 0.05$]) (Figure 2a). When corrected for macrophage numbers in the lining layer (determined by CD68 staining), the difference in the percentages of TNFα-positive cells was not significant, suggesting that macrophage numbers could account for the differential expression observed. Preincubation of the primary anti-TNFα mAb with recombinant human TNFα effectively neutralized the staining, confirming antibody specificity (data not shown).

IL-1 expression. IL-1α and IL-1β were easily detected in the PsA SM lining layer, with a median ± SD of 17.3 ± 12.7% IL-1α–positive cells and 11.0 ± 10.1% IL-1β–positive cells (Figures 1b and 2b and c). Lower percentages of IL-1α– and IL-1β–expressing cells were noted in sublining areas (8.9 ± 7.1% IL-1α–positive cells/hpf and 7.6 ± 6.2% IL-1β–positive cells/hpf, respectively), localized in particular to perivascular areas. A subset of tissues showed intense IL-1β staining in endothelial cells (~50% of the biopsy samples). Such tissues, however, did not exhibit increased vascular scores and were present in samples from both RA and PsA patients. Although no difference was noted for IL-1α staining in PsA SM versus RA SM (Figure 2b), IL-1β staining was seen at significantly higher levels in RA lining layer (24.6 ± 13.3% positive cells/hpf; $P = 0.02$ versus PsA lining layer) (Figure 2c). After correction for macrophage numbers using CD68 staining, the difference in IL-1β lining layer staining between PsA and RA SM was no longer significant, again indicating that macrophage numbers could account for the differences observed.

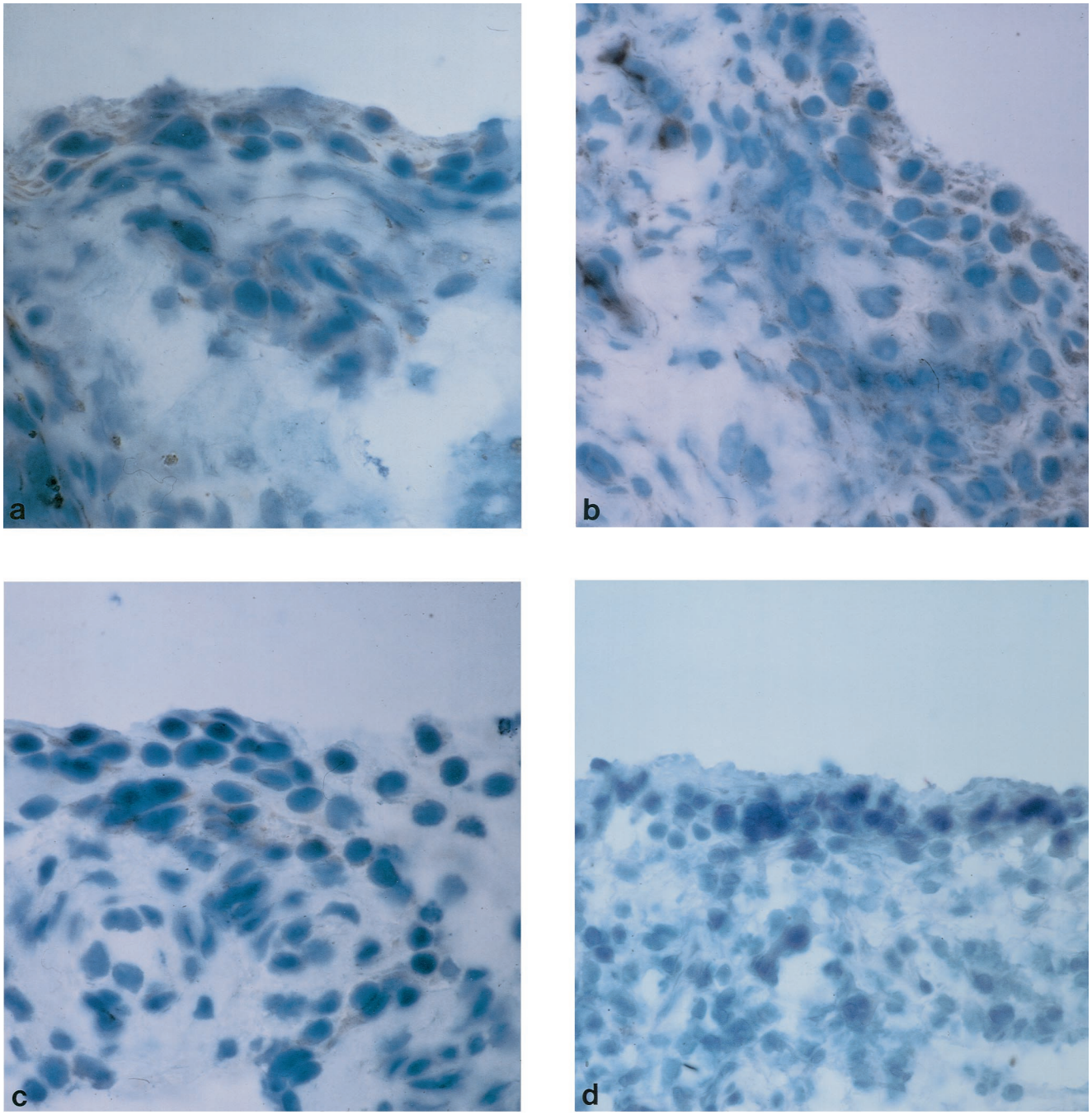


Figure 1. Cytokine expression in psoriatic arthritis (PsA) synovial membrane (SM). PsA SM sections were stained with monoclonal antibodies directed against human tumor necrosis factor α (**a**), interleukin-1β (IL-1β) (**b**), IL-15 (**c**), and a nonspecific IgG negative control (**d**). Positive cells were detected using diaminobenzidine substrate (brown). (Original magnification $\times$ 600.)

IL-15 expression. IL-15 is a recently recognized pleiotropic cytokine that is capable of T cell and macrophage activation (15,16). IL-15 expression in the absence of IL-2 has recently been demonstrated in RA SM (17–19), but has not been previously reported in PsA SM. We detected IL-15 in PsA SM, the expression being more prominent in the lining layer (median $\pm$ SD 7.3 $\pm$ 8.7% positive cells/hpf) than in the sublining tissue (3.5 $\pm$ 4.5% positive cells/hpf) (Figures 1c and 2d). Staining of endothelial cells was occasionally noted. Of

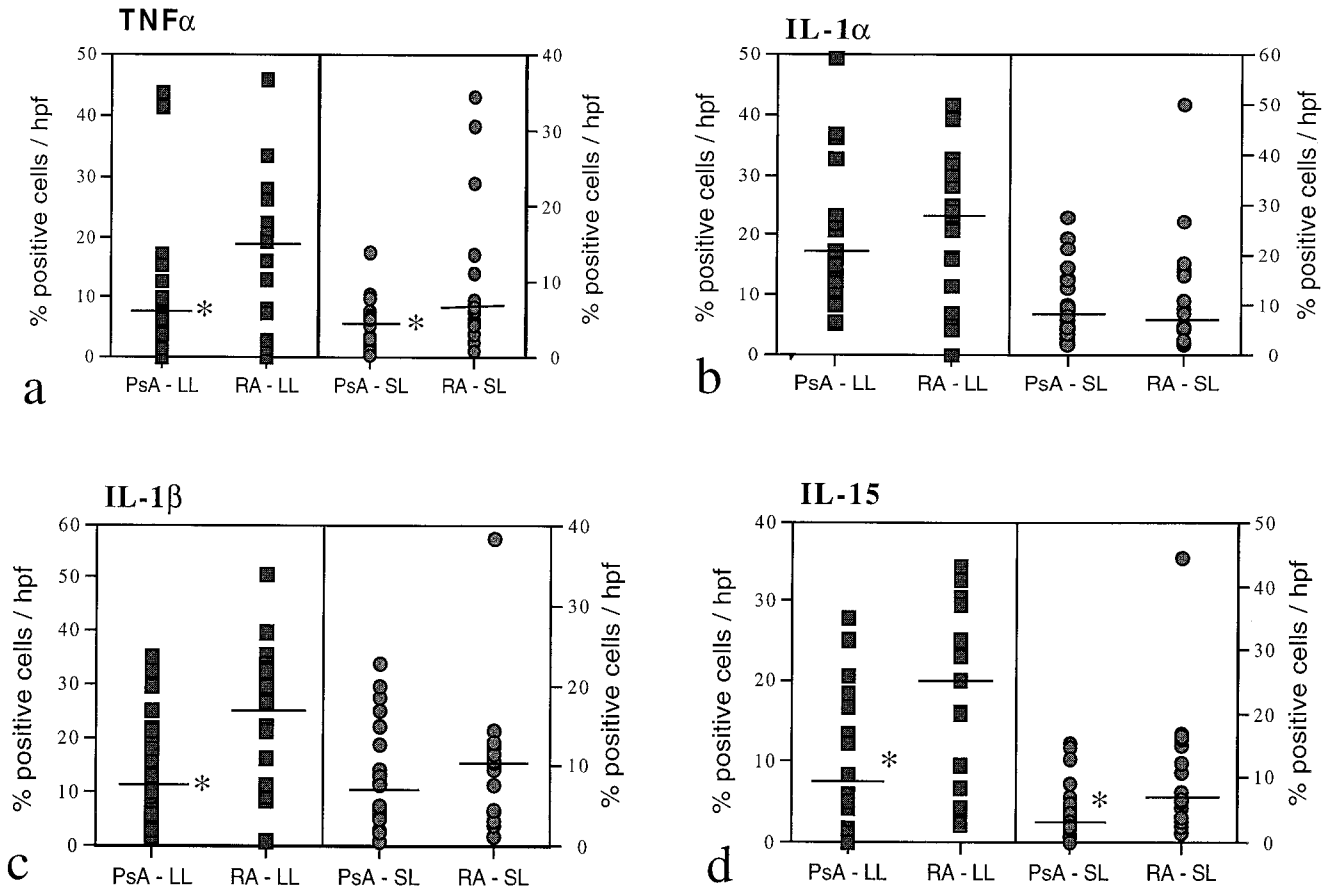
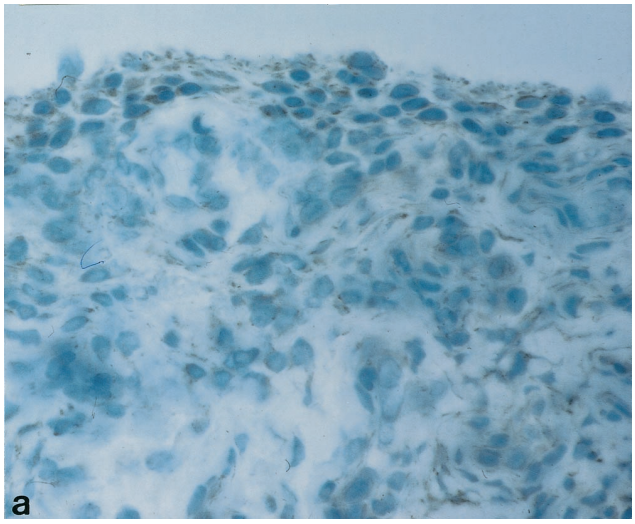


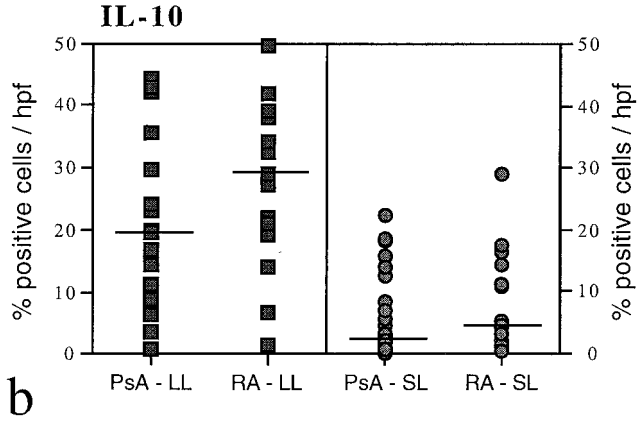
Figure 2. Comparison of the expression of the cytokines tumor necrosis factor α (TNFα) (**a**), interleukin-1α (IL-1α) (**b**), IL-1β (**c**), and IL-15 (**d**) in the lining layer (LL) and sublining layer (SL) of psoriatic arthritis (PsA) versus rheumatoid arthritis (RA) synovial membrane. Each data point represents the mean percent positive cells per high-power field (hpf) for a given patient. Bars represent group medians. $* = P < 0.05$.

interest, there was less IL-15 in PsA SM than in RA SM, in which there were 20.0 ± 11.2% positive cells/hpf in the lining layer ($P = 0.04$) and 6.5 ± 10.3% positive cells/hpf in the sublining ($P = 0.02$) (Figure 2d). As was found with TNFα and IL-1β, differences in lining layer IL-15 expression were no longer significant after correction for lining layer CD68 expression. IL-15 staining was completely abrogated by preincubation of anti–IL-15 mAb with recombinant human IL-15 (data not shown).

IL-10 expression. The presence of the antiinflammatory cytokine IL-10 was detected in both the lining layer and sublining of PsA SM. The percentage of positively stained cells was higher in lining layer than in sublining areas (mean ± SD 19.6 ± 13.7% positive cells/hpf versus 2.0 ±7.1% positive cells/hpf). These levels were not significantly different from those found in RA SM (lining layer 28.1 ± 13.7% positive cells/hpf, sublining 4.5 ± 8.2% positive cells/hpf) (Figure 3b). Interestingly, expression of IL-10 did not correlate with expression of TNFα in the synovial lining layer, but expression of these 2 cytokines did correlate in the sublining tissue (r = 0.4, $P = 0.04$). The median ± SD ratio of TNFα:IL-10 was not significantly different in the lining layer of PsA SM versus RA SM (0.5 ± 1.6 and 0.6 ± 0.4, respectively). However, in the sublining areas, this ratio was higher in PsA SM than RA SM (2.7 ± 3.5 versus 1.0 ± 1.9; $P = 0.03$) (Figure 3c).

NF-κB expression. There is evidence that proinflammatory cytokines, such as TNFα and IL-1, act through NF-κB–dependent pathways, and that such pathways play important roles in synovial cell proliferation, leukocyte infiltration, and further proinflammatory cytokine production (20,21). We sought to determine if the differences in cytokine expression in SM of RA patients and PsA patients led to an overall difference in the extent of NF-κB activation in these tissues. To this





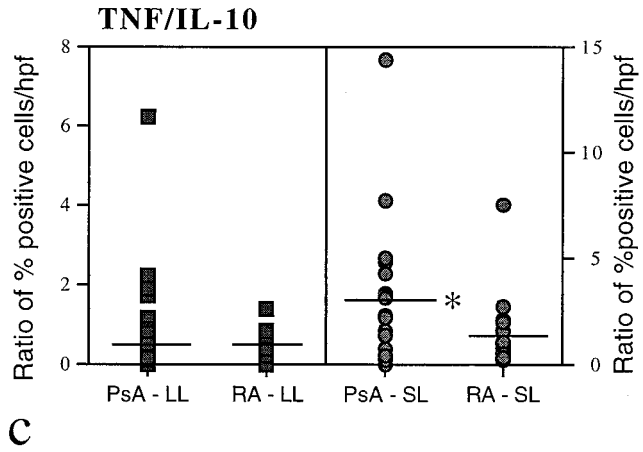


Figure 3. IL-10 expression in PsA versus RA synovial membrane (SM). **a,** PsA SM section stained with monoclonal antibody directed against IL-10. Positive cells were detected using diaminobenzidine substrate (brown) (original magnification × 400). **b,** Comparison of the expression of IL-10 in the lining layer and sublining layer of PsA versus RA SM. Each data point represents the mean percent positive cells per high-power field for a given patient. Bars represent group medians. **c,** Comparison of TNFα:IL-10 ratios in the lining layer and sublining layer of PsA versus RA SM. $* = P = 0.03$. See Figure 2 for other definitions.

end, staining with an antibody specific for the activated subunit, p65, of NF-κB was quantified. Within the lining layer, nuclear and cytoplasmic staining of activated NF-κB was noted in PsA SM (Figure 4b), in amounts that were less than, but not significantly different from, those in RA SM (median ± SD 35.6 ± 14.0% and 47.1 ± 15.4% positive cells/hpf, respectively). In the sublining, however, 12.2 ± 11.3% cells/hpf were positive in PsA SM, compared with higher levels of expression in RA SM (26.6 ± 14.2%; $P = 0.008$) (Figure 4a). Within the sublining areas, expression of activated NF-κB correlated significantly with expression of TNFα ($r = 0.54$, $P = 0.001$), but similar correlations were not seen in the lining layer, nor for IL-1 expression with activated NF-κB in any area.

Cytokine expression in skin. Diffuse TNFα, IL-1α, and IL-1β expression was evident in the basal areas of the epidermis and in dermis of lesional skin (Figures 5a and b), primarily localized around blood vessels and, in some cases, including endothelial cells. Levels of cytokine expression were comparable with those found in the sublining areas of PsA SM, with median (±SD) values within the dermis as follows: TNFα 3.8 ± 3.5% positive cells/hpf, IL-1α 6.2 ± 7.2% positive cells/hpf, IL-1β 11.6 ± 5.6% positive cells/hpf. Interestingly, perilesional skin tended to have higher levels of dermal expression of these cytokines, but the difference reached statistical significance only for TNFα (6.3 ± 5.9% positive cells/hpf; $P = 0.02$). IL-15 production by keratinocytes has been reported, with postulated regulation by exposure to ultraviolet light (22,23). In the present study, evaluation of the dermal expression of IL-15 in lesional skin revealed levels that were comparable with those seen in sublining regions of PsA SM (2.6 ± 4.5% positive

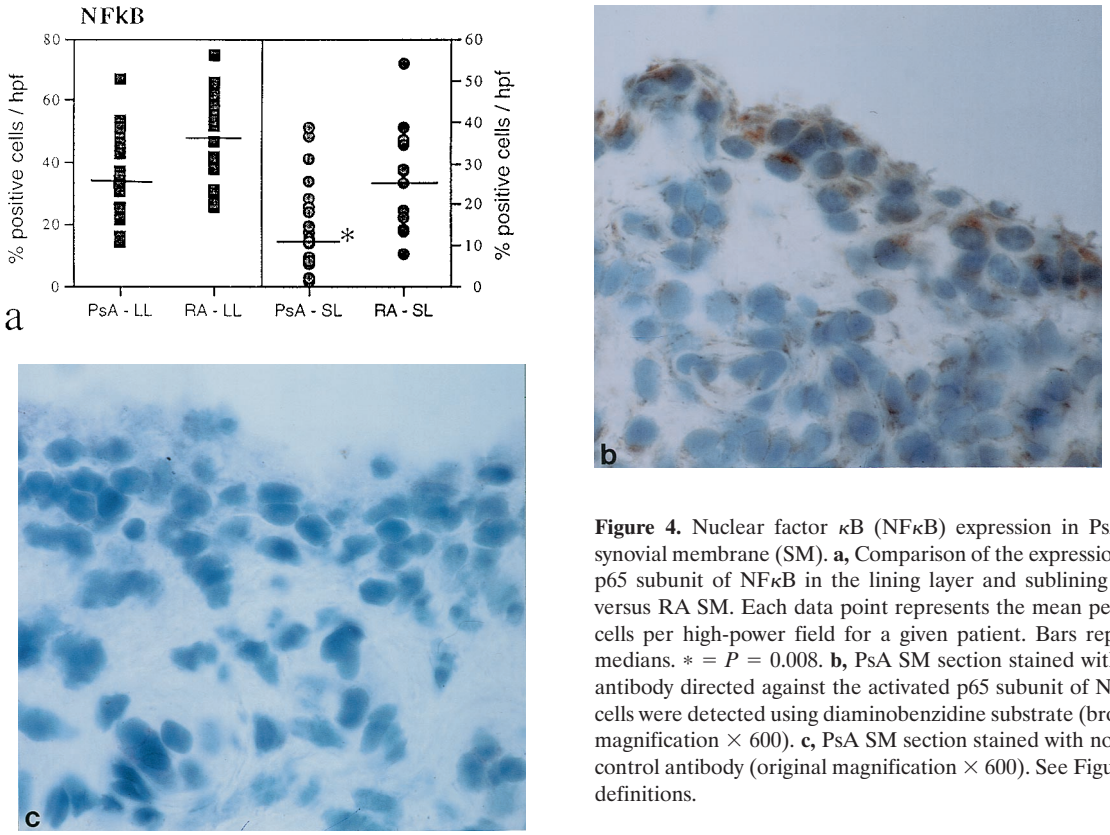


Figure 4. Nuclear factor κB (NFκB) expression in PsA versus RA synovial membrane (SM). **a,** Comparison of the expression of activated p65 subunit of NFκB in the lining layer and sublining layer of PsA versus RA SM. Each data point represents the mean percent positive cells per high-power field for a given patient. Bars represent group medians. $* = P = 0.008$. **b,** PsA SM section stained with monoclonal antibody directed against the activated p65 subunit of NFκB. Positive cells were detected using diaminobenzidine substrate (brown) (original magnification × 600). **c,** PsA SM section stained with nonspecific IgG control antibody (original magnification × 600). See Figure 2 for other definitions.

cells/hpf) but were significantly less than those found in the lining layer of PsA SM ($P = 0.008$). Perilesional skin was also noted to have higher levels of IL-15 expression in the dermis than lesional skin (4.5 ± 5.7% positive cells/hpf; $P = 0.003$).

In contrast to the proinflammatory cytokines, IL-10 expression in the dermis of psoriatic lesions was significantly less than in PsA SM (median ± SD 1.6 ± 2.6% positive cells/hpf; $P = 0.01$ versus sublining of PsA SM). This implies that a relative deficiency of IL-10 may play a more important role in psoriatic lesional skin than in PsA SM. Perilesional skin was noted to have marked basal keratinocyte staining of IL-10, which was not present in lesion samples (Figures 5d and e). In perilesional dermis, IL-10–positive cells were comparable in number with those in lesional skin (1.9 ± 2.1% positive cells/hpf). This indicates that a relative reduction of dermal IL-10 expression may occur early in the development of the lesion.

Although quantitative similarities were found in cytokine expression in lesional skin and SM of PsA patients (Figure 6), no consistent direct correlations were found between the 2 sites of inflammation.

Comparison with cytokine expression in OA synovial membrane. As a further control, we also investigated cytokine expression in OA synovial tissues. As expected, levels of TNFα, IL-1α, and IL-1β expression were significantly higher in PsA SM than in OA SM, with mean cytokine staining of <2% in the OA SM sample group in both the lining layer and sublining areas.

DISCUSSION

The present study represents the first systematic analysis characterizing the relative quantity and localization of pro- and antiinflammatory cytokines, as well as activation of the downstream transcription factor NF-κB, in PsA SM and skin. We have shown that key mediators of inflammation, including TNFα, IL-1α, IL-1β, and IL-15, are present in both SM and skin of patients with PsA, although levels of each cytokine do

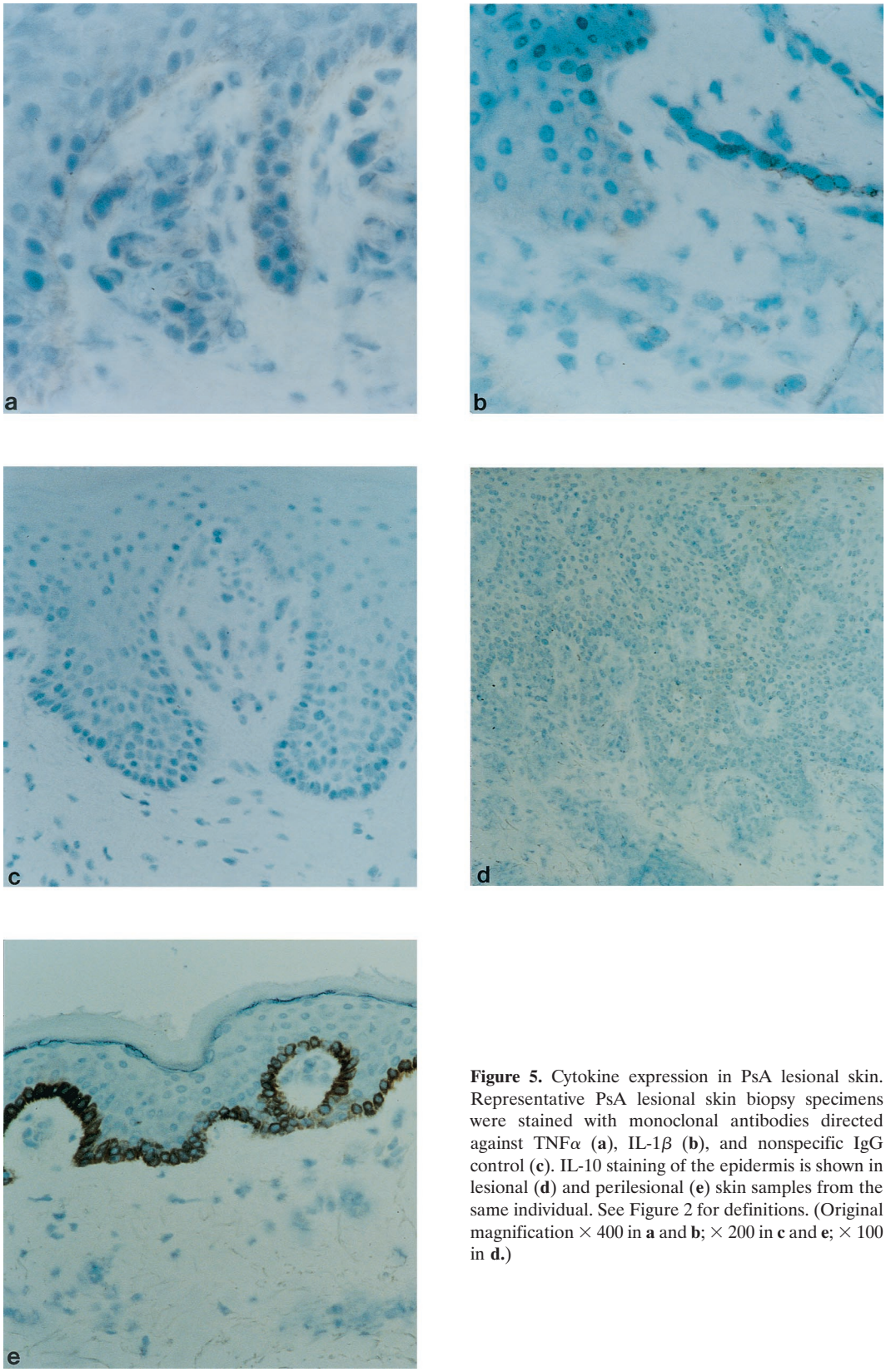


Figure 5. Cytokine expression in PsA lesional skin. Representative PsA lesional skin biopsy specimens were stained with monoclonal antibodies directed against TNFα (**a**), IL-1β (**b**), and nonspecific IgG control (**c**). IL-10 staining of the epidermis is shown in lesional (**d**) and perilesional (**e**) skin samples from the same individual. See Figure 2 for definitions. (Original magnification $\times$ 400 in **a** and **b**; $\times$ 200 in **c** and **e**; $\times$ 100 in **d.**)

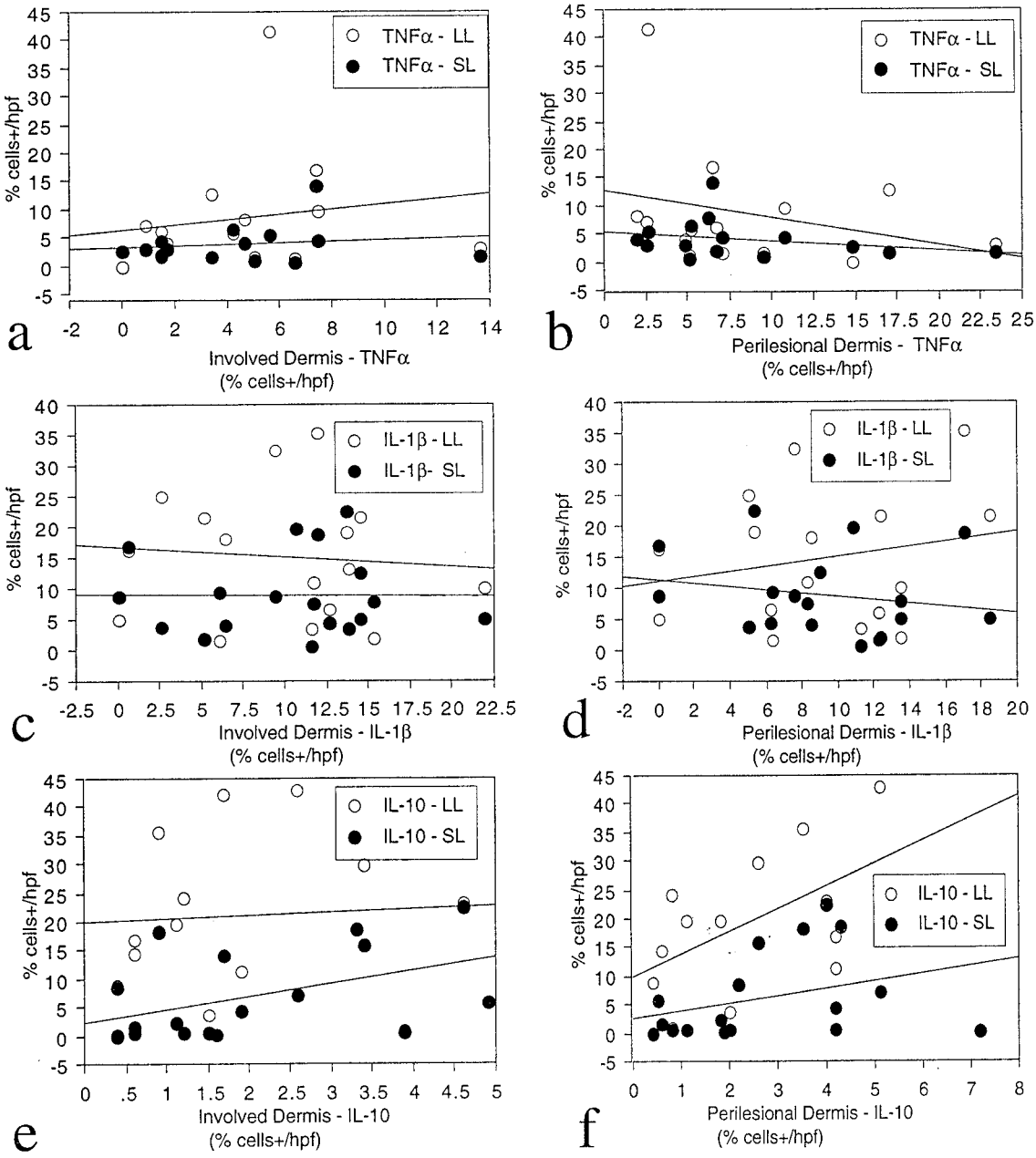


Figure 6. Correlations of cytokine expression in PsA synovial membrane (SM) versus lesional or perilesional PsA skin. Comparison of TNFα (**a** and **b**), IL-1β (**c** and **d**), and IL-10 (**e** and **f**) expression in PsA SM with expression in lesional (**a, c,** and **e**) and perilesional (**b, d,** and **f**) skin samples from matched patients. Each data point represents the mean percent positive cells per high-power field in the subepidermal dermis versus the lining layer or sublining layer of SM, for a given patient. See Figure 2 for other definitions.

not necessarily correlate directly between the 2 tissue sites. Given the therapeutic success of inhibiting TNFα and IL-1 in RA (1,2,24), we also compared cytokine expression in PsA SM samples with that in samples from a clinically matched cohort of patients with established RA. Tissue morphology was similar between PsA and RA SM, except for a relative decrease in macrophage numbers in PsA samples. Consistent with the detection

of a lower proportion of lining layer macrophages, fewer IL-1β–positive cells were noted in the lining layer of PsA SM as compared with RA SM, and less TNFα and IL-15 was noted in both lining layer and sublining areas of PsA SM. No differences in IL-10 levels were noted. Furthermore, fewer cells expressing activated NF-κB were found in the sublining layer of PsA SM than in that of RA SM. These quantitative differences in cell subset distribution and cytokine expression suggest that there may be subtle but potentially important differences in the immune responses in these 2 inflammatory arthritides.

The clinical efficacy of TNFα and IL-1 inhibition illustrates the importance of these cytokines in the inflammatory response in RA (1–3,24). The comparable expression of these cytokines observed in the present study suggests that such mediators of inflammation are also significant in PsA. The decreased numbers of TNFα- or IL-1β–positive cells in the lining layer may be explained, in part, by the reduced macrophage numbers found as compared with RA. Decreased macrophage infiltration in PsA SM has been reported previously (12). However, numbers of CD14+ macrophages, presumably representing recently recruited cells and from which monokine secretion might be predominantly expected, were equivalent in PsA and RA synovial lining layer in our study. Furthermore, TNFα, IL-1β, and IL-15 expression, when corrected for CD14 expression, remained lower in PsA than that seen in RA lining layer.

Irrespective of whether macrophages intrinsically produce less cytokine, our results now clearly demonstrate lower levels overall of macrophage-derived cytokines present in PsA tissue. This is compatible with previous data showing lower amounts of TNFα and IL-1β in the synovial fluid of PsA patients as compared with that of RA patients (13). Conversely, higher levels of TNFα and IL-1β production by cultured psoriatic synovium compared with dermal or RA synovial explants have been reported (25). It is possible that these apparently contradictory data reflect differences in patient characteristics or experimental systems utilized. In the previous study, neither articular distribution nor disease severity was documented (25). Moreover, aggressive immunosuppressive therapy among RA patients may account, at least in part, for the relatively low level of cytokine production observed in that study. Our cohort of PsA patients was skewed in that the vast majority had polyarticular, erosive arthritis, and none of these patients were receiving standard immunosuppressive agents other than low-dose prednisone. Indeed, since more patients in our RA group were taking disease-modifying antirheumatic drugs/corticosteroids than in the PsA group, the differences seen in proinflammatory cytokine expression may actually be an underestimate. The antiinflammatory effects of methylprednisolone on synovial inflammation have been documented previously (26). Nevertheless, we recognize that caution should be exercised in interpreting the results of histologic examination of in vivo dynamic cytokine production.

TNFα and IL-1 can activate NF-κB, leading to synovial cell proliferation, leukocyte trafficking, and further proinflammatory cytokine production (20,21). Since expression of TNFα and IL-1 was lower in PsA SM than in RA SM, we investigated the possibility that this might be reflected in lower levels of NF-κB activation. Activated NF-κB has been shown to be present in both lining layer and sublining of RA SM (27–29). As in RA SM, the activated p65 subunit of NF-κB was present in PsA SM, and, as with TNFα, the level expressed was less than that in RA SM, particularly in the sublining regions. Furthermore, the number of TNFα-positive cells correlated with the percentage of cells expressing activated NF-κB in the sublining area. While the presence of activated NF-κB confirms that TNFα and IL-1 are functionally active in PsA SM, the biologic significance of this divergence from RA SM is unclear. Our data suggest that, although TNFα and IL-1 appear part of a common synovial inflammatory response, quantitative variations exist, which likely reflect distinct etiologies, in turn implying clinically disparate phenotypes.

IL-15 may also activate NF-κB (30). The potential importance of this pleiotropic cytokine in RA has recently been recognized (15,17,18). The present findings now reveal its expression in PsA synovium and skin. The presence of the related 4–α-helix family cytokine, IL-2, has been described in PsA SM, but not RA SM (19). Moreover, we detected higher levels of IL-15 in RA than in PsA synovium, suggesting cross-regulation between these 2 cytokines. Several functional interactions between IL-15 and IL-2 have been reported, since they share common receptor β and γ chains. IL-15 can amplify IL-2–mediated responses, while down-regulating its own high-affinity–binding IL-15 receptor α chain (31,32). Although both cytokines can activate T cells and lead to proliferation, there are differences in the degree of these responses (33). In inflammatory bowel disease, up-regulation of IL-15 is reported in the peripheral blood and lesions of patients with active ulcerative colitis, in which IL-2 levels are low, but IL-15 is detected to a lesser extent in Crohn's disease, where IL-2 is present (34).

A comparable difference may exist between PsA

and RA in that IL-15 may play a more dominant role in RA, whereas IL-2 may be more significant in PsA. The functional significance of such a dichotomy in terms of T cell activation is unclear at present, but it may suggest that T cells, through IL-2, and macrophages, through IL-15, may differentially regulate T cell activation in PsA and RA, respectively. For example, IL-15 (but not IL-2) can stimulate formation of osteoclast-like cells (35), and RA patients, unlike PsA patients, are more likely to demonstrate diffuse osteopenia with no new bone formation. The factors that regulate differential IL-2 and IL-15 expression in synovial tissues in either condition are currently unclear and require further investigation.

The presence and functional importance of IL-10 has been previously demonstrated in RA (36–38). IL-10 potentially down-regulates macrophage and T cell function and may favorably modify metalloproteinase:tissue inhibitor of metalloproteinases ratios in inflammatory tissues (39,40). In the present study, we found no significant differences in the amount of IL-10 expressed in PsA SM as compared with RA SM. The biologic consequences of pro- versus antiinflammatory cytokine expression may be reflected in a ratio rather than as absolute numbers (41). When we evaluated the TNFα: IL-10 ratio in PsA SM versus RA SM, we found that this ratio was slightly higher in sublining of PsA SM. This did not correlate with higher levels of activated NF-κB in PsA SM, suggesting that other proinflammatory cytokines in SM, such as IL-1β and IL-6, also contribute to the increased NF-κB activation seen in RA SM compared with PsA SM.

In addition, histologic findings do not readily reflect the influence of soluble inhibitors, e.g., TNF receptors or IL-1 receptor antagonist. Thus, the increased TNFα:IL-10 ratio in PsA SM most likely indicates that a relative paucity of IL-10 may play a significant role in PsA and implies that IL-10 supplementation may have therapeutic efficacy similar to, or perhaps even greater than, that reported in RA (42). Interestingly, IL-10–positive cells were less frequent in lesional psoriatic skin than in PsA synovium, suggesting that biologic therapies should not be expected a priori to exert equivalent efficacy at different tissue sites in PsA. Reduced dermal IL-10 expression at the messenger RNA level in lesional skin plaques has been reported (43). Moreover, IL-10 administration suppressed Th1 responses and induced clinical improvement in injected lesions. Findings of the present study support the rationale for use of IL-10 to treat both skin and joint involvement in PsA.

In summary, understanding dominant cytokine pathways in PsA is critical to better comprehension of pathogenesis and to development of novel, effective therapies. Common pathways in the synovial immune responses in PsA and RA, such as the presence of TNFα, IL-1, and activation of NF-κB, are evident, but quantitative differences exist, suggesting diverse upstream stimuli and regulatory influences. In PsA, the lower number of lining layer macrophages, the potential predominance of IL-2 over IL-15, and previous data supporting the notion of oligoclonal T cell expansion clearly suggest that, compared with RA, PsA is more likely a T cell–dependent, antigen-driven disease (8,44). This appears to be the case for the skin as well as the synovium, although the 2 tissues may manifest discrete mechanisms whereby constituent cells and cytokines perpetuate inflammation. Nevertheless, even if inciting events in PsA and RA are distinct, the present results clearly demonstrate that synovial responses ultimately share common pathways including, at least, TNFα and IL-1, and likely other mediators of inflammation. Thus, novel biologic strategies such as IL-10 supplementation or TNFα and IL-1 blockade, shown to be effective in RA, may have utility in PsA as well.

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